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Collection and characterization of wild Basidiomycetes from the district Ludhiana (Punjab)

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Abstract

Survey of field and forest area of district Ludhiana was conducted to explore diversity of wild mushrooms for their collection, identification and characterization. Five mushrooms *Chlorophyllum molybdites*, *Agaricus* sp., *Agaricus silvicola*-a, *Agaricus silvicola*-b and *Agaricus arvensis* were collected from the district Ludhiana which were accessioned as DMRO-568, DMRO-569, DMRO-570, DMRO-571, DMRO-572, respectively at Directorate of Mushroom Research, Chambaghat, Solan (India). Identification was made on the basis of mushroom morphology and microscopic characteristics. Linear growth on Complete Yeast extract Medium agar up to 10 days of incubation showed maximum growth for culture *Agaricus arvensis* (DMRO-572) as 8.00mm/d on 4th day while biomass was the maximum by *Chlorophyllum molybdites* (DMRO-568) as 2.48g/L/d. Maximum exoglucanase activity was seen in *Agaricus silvicola*-a (DMRO-570) (1.468 U/mg proteins) and endoglucanase activity in *Agaricus silvicola*-b (DMRO-571) (1.622 U/mg proteins). Xylanase activity was maximum for *Agaricus silvicola* (DMRO-570) (0.779 U/mg proteins). Laccase activity was observed maximum in *Agaricus* sp. (DMRO-569) and *Agaricus arvensis* (DMRO-572) (5.25 U/mg proteins). Spawn production of these cultures on wheat grains showed better growth by *Agaricus arvensis* (DMRO-572) as 4.25mm/d, 3.69mm/d and 5.66mm/d on 8th, 16th and 24th day, respectively. Compost was the preferred substrate for *Chlorophyllum molybdites* (DMRO-568) while wheat straw and paddy straw for *Agaricus arvensis* (DMRO-572). Four different identified species related to well known genus *Agarics* could be exploited for their commercial potential, but *Chlorophyllum molybdites* was inedible.

Key words – *Agaricus* species − characterization − *Chlorophyllum molybdites* − diversity − Spawn preparation − substrate selection

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Introduction

In India, parts of which are considered as biodiversity hotspots, only 27,000 species of fungi have been recorded (Chang & Miles 2004, Singh 2011). If the fungus-plant ratio of 6·1 proposed for the tropics is applied, the number of fungal species in India can be estimated as greater than 250,000 (colonizing approximately 42,000 plants species known to occur) (Sankaran 2013). This means that hardly 10% of the country's fungi are known till date. Northern India represents a characteristic vegetation of Indian subcontinent which is noticeable both in quality and quantity because of different latitudinal, altitudinal and habitat conditions. Its humid climatic conditions, plant distributions and field features are suitable for the growth of macrofungi. Six hundred and fifty species of *Agarics, Polyporus* (159 species), *Fomes* (59 species), *Trametes* (35 species), *Poria* (27 species), 55 Taxa of *Russula* and 26 taxa of *Lactaricus* have been reported from India (Manoharachary et al. 2005). So motives of Survey and Conservation are: (i) Collection of edible fruiting bodies (ii) Great aesthetic value (iii) Biodiversity conservation by preserving some habitats of fungi (iv) Conserved Saprobe fungi leading to decomposition increment of litter accumulation disturbed nutrient cycles.

Punjab harbors in North-East India represent diversified agro-climatic zones; which could provide diversity in wild mushroom flora. Fungal diversity in forest area of Punjab could be explored to collect edible wild mushroom species with genetical and nutraceutical vigour. District Ludhiana is located at 30.9°N 75.85°E having altitudinal elevation of 244m (798 ft) and occupied 3767 km^2 area of Northern India. Keeping the importance of macrofungi on priority, survey of field and forest area of the district Ludhiana was conducted for collection, identification and characterization of wild mushroom flora.

Materials & Methods

Collection of wild mushrooms

During survey of field and forest area when new mushroom was found in their natural habitat, its photograph was taken and GPS location was noted down along with nearby village or town's name. After that, carefully dig out the mushroom, put it into the sterilized sample polybag and carried it into sample box. Mushroom with unopened cap was preferred and taken in duplicate or triplicate form (if possible). Soil sample of the mushroom growing area was also collected (if it grows on soil) and brought to Mycology Lab and Mushroom Research Complex, PAU, Ludhiana, for further analysis.

Morphological identification

These mushroom samples were identified on the bases of their macroscopic and microscopic characters. Macroscopic characters included pileus and stipe size, color, shape and margin, along with microscopic characters gill attachment to stipe, color, density, spore color and size. There are different types of pileus shapes, these are campanulate (bell shaped), conical (triangle), convex (outward rounded), depressed (with a low central region), flat (with atop of uniform height), infundibuliform (deeply depressed funnel shaped), ovate (shape like half an egg), umbllicate (with a small deep depression) and umbonate (with a central bump or knob). There are also different type of stipe according to their shape stipe can be equal, clavate or club shaped, ventricose or swollen, bulbous, fusoid and radiating. The way that gills attach to the top of the stalk is an important feature of mushroom morphology. Mushrooms in the genera *Agaricus, Amanita, Lepiota* and *Pluteus*, among others, have free gills that do not extend to the top of the stalk. Others have decurrent gills that extend down the stalk, as in the genera *Omphalotus* and *Pleurotus*. There are a great number of variations between the extremes of free and decurrent, collectively called attached gills. Finer distinctions are often made to distinguish the types of attached gills: adnate gills, which adjoin squarely to the stalk; notched gills, which are notched where they join the top of the stalk; adnexed gills, which curve

upward to meet the stalk, and so on. In the *Basidiomycetes*, usually four spores develop on the tips of thin projections called sterigmata which extend from a club shaped cell called a basidium. The most important microscopic feature for identification of mushrooms is the spores themselves. Their color, shape, size, attachment, ornamentation, and reaction to chemical tests can be the crux of identification. Spores often have a protrusion at one end, called an apiculus, which is the point of attachment to the basidium, termed the apical germ pore from which the hypha emerges when the spore germinates. The cultures prepared from wild mushrooms by tissue culture technique on Potato dextrose agar (PDA) slants, were deposited and accessioned at Directorate of Mushroom Research, Chambaghat, Solan.

Physicochemical analysis of soil

Soil samples from the surrounded area of respective mushroom were analyzed for their physicochemical properties by using standard methodology of Soil testing in India (STI 2011). Soil texture was estimated by using standard pyramid between clay, silt and sand ratio. Soil moisture was calculated by using Gravimetric method (Black 1965). pH estimation was done by using pH meter. Carbon content was measured by muffle burning method. Kjeldahl method was used to estimate total nitrogen content of soil (AOAC 1995). Method used for determination of available phosphorus in soil was Olsen's Method for neutral and alkaline soils (Olsen et al. 1954). Potassium estimation in soil samples was done by Flame photometric method (Toth & Prince 1949).

Characterization of wild mushroom cultures

Linear growth and biomass production studies:

Linear growth study of wild mushroom cultures was observed on Complete yeast extract agar (CYM) at 25$\pm$2°C up to 10th day of incubation. Biomass production capability of wild mushroom cultures was also observed in Complete yeast extract broth. Each flask contained 50ml Complete yeast extract broth was inoculated with mycelial agar bit (5 mm dia) and weight of biomass was taken after 5 and 10 days of incubation. Wet weighed biomass was kept at 55^{o}C up to 4 hours and weighed as dry weight by subtracting out the dry weight of filter paper.

Enzyme activity study:

Enzyme activity was assayed by growing the mycelium in Mushroom minimal medium broth (MMM). Flasks with MMM broth were inoculated with agar bits of the wild mushroom cultures from the master plates and incubated at 30 ± 2°C for 10 days. Each flask was filtered after incubation period of 5 and 10 days on to Whatmann No. 1 filter paper. The filtrate was collected in capped vials, stored at 4°C and used to estimate the enzyme activity. Cellulases (exoglucanase & endoglucanase) and xylanases were assayed by method of Sandhu & Kalra (1982). Laccase was assayed by method of Dhaliwal et al. (1991). Absorbance was read at 540 nm for cellulases and xylanase, but at 495 nm for laccase, using UV-visible spectrophotometer (Elico SL-164). The amount of reducing sugars released was estimated using glucose standard curve. Total proteins estimation was done by Lowry's method (1951). Specific enzyme activity was calculated by dividing respective enzyme activity to the total proteins estimated from that sample.

Specific Activity = Relative Activity (U/mL) / Protein Concentration (mg/mL)
= U/mg proteins

Spawn preparation

For spawn preparation, wheat grains were boiled in water for 30 min (wheat grains: water, 1:2 w/v) so as to cook them soft enough to be pressed within the fingers. Extra water was sieved out. The grains were cooled and mixed with 1:2 ratios of calcium carbonate and calcium sulphate powder and then filled in the spawn tubes (25 mm × 198 mm) to a height of approximately 150 mm, plugged with non- adsorbent cotton. Autoclaved tubes were inoculated with mylcelial agar bit of each sample and incubated at 25$\pm$2°C and mycelia run rate was recorded as mm/d for 8,16 and 24 days.

Substrate selection

Three substrates Compost, Wheat straw and Paddy straw were selected. Compost prepared by short method using standard methodology by Punjab Agricultural University (Khanna & Kapoor 2007). Wheat straw and paddy straw were ground up to approximately 0.5 mm size and overnight soaked in water. All the substrates were taken into glass petri dishes by making a uniform bed and sterilized them at 20 lbs for 90 min. The substrates were inoculated with mycelia agar bit (5 mm dia) of each wild mushroom culture and incubated at 25±2°C. Growth rate was observed on 8^{th}, 16^{th} and 24^{th} day as mm/d.

Statistical analysis

Statistical analysis was done by CPCS1 software developed by the Department of Mathematics, Statistics and Physics, Punjab Agricultural University, Ludhiana, Punjab and WASP 1.0 developed by ICAR GOA Research complex, India.

Results

Description of wild mushrooms and physicochemical analysis of soil collected from field and forest area of the district Ludhiana

Chlorophyllum molybdites Fig. 1a

This wild mushroom was collected from village Ayali Kalan, district Ludhiana, from the roadside leaf litter area in July 2013. The GPS location was recorded as 30° 53' 40" N, 75°45'37" E. A sample of the soil around the mushroom was collected and analyzed for physicochemical properties. The soil was assessed as sandy-loam containing clay, silt and sand as 8.00, 16.6 and 53.8%, respectively. The soil pH and moisture were 7.5 and 54%, respectively. The C, N, P, K content was also measured along with $CaCO_3$ and Fe_2O_3, in which carbon content was 5.2 mg/g of soil and nitrogen content was 1.3 mg/g of soil (Table 2). The mushroom had a typical smell but no taste. The pileus was cream colored with 110 mm diameter which was round and flattened at top. The pileus was non-sticky, non-hygrophanous and bearing scales. The stipe was centrally attached, which was 205mm long. Annulus type ring was present, but veil and volva were absent (Table 1). Gills underneath the pileus were free, light greenish colored giving sea green spores on filter paper.

***Agaricus* sp.** Fig. 1b

This wild mushroom was collected from the forest area of district Ludhiana, near village Hambran on GPS location 30°56'20" N 75°40'13" E, during the month of August, 2013. The mushroom was collected from decomposed cattle dung mixed with soil. Soil around the mushroom was collected and analyzed for physicochemical properties. The soil was assessed as clayey loam containing clay, silt and sand as 9.20, 15.7 and 51.3%, respectively. The soil pH and moisture were 7.3 and 57%, respectively. The C, N, P, K content was also measured along with $CaCO_3$ and Fe_2O_3, from which carbon content was 5.9 mg/g and nitrogen content was 1.5 mg/g of soil (Table 2). The pileus was creamish brown with 23mm diameter; it was ovate in shape and curved along the margin without scales. The pileus surface was non-sticky and non-hygrophanous in nature. The centrally attached stipe was also creamish colored with 38 mm in length, slender shape and swollen base. An annulus type ring was present on the stipe near to pileus but veil and volva were not present (Table 1). The lamellae underneath the pileus were freely attached to stipe with pinkish coloration which later on maturation gave brown spores on filter paper.

Agaricus silvicola Fig. 1c

It was the wild mushroom collected from leaf litter surrounded by grass at village Ladowal, district Ludhiana on GPS location 30° 59' 28N and 75° 44' 10" E during the month of September, 2013. Soil around the mushroom was collected and analyzed for physicochemical properties. The soil was assessed as sandy loam containing clay, silt and sand as 8.70, 15.2 and 54.5%, respectively. The

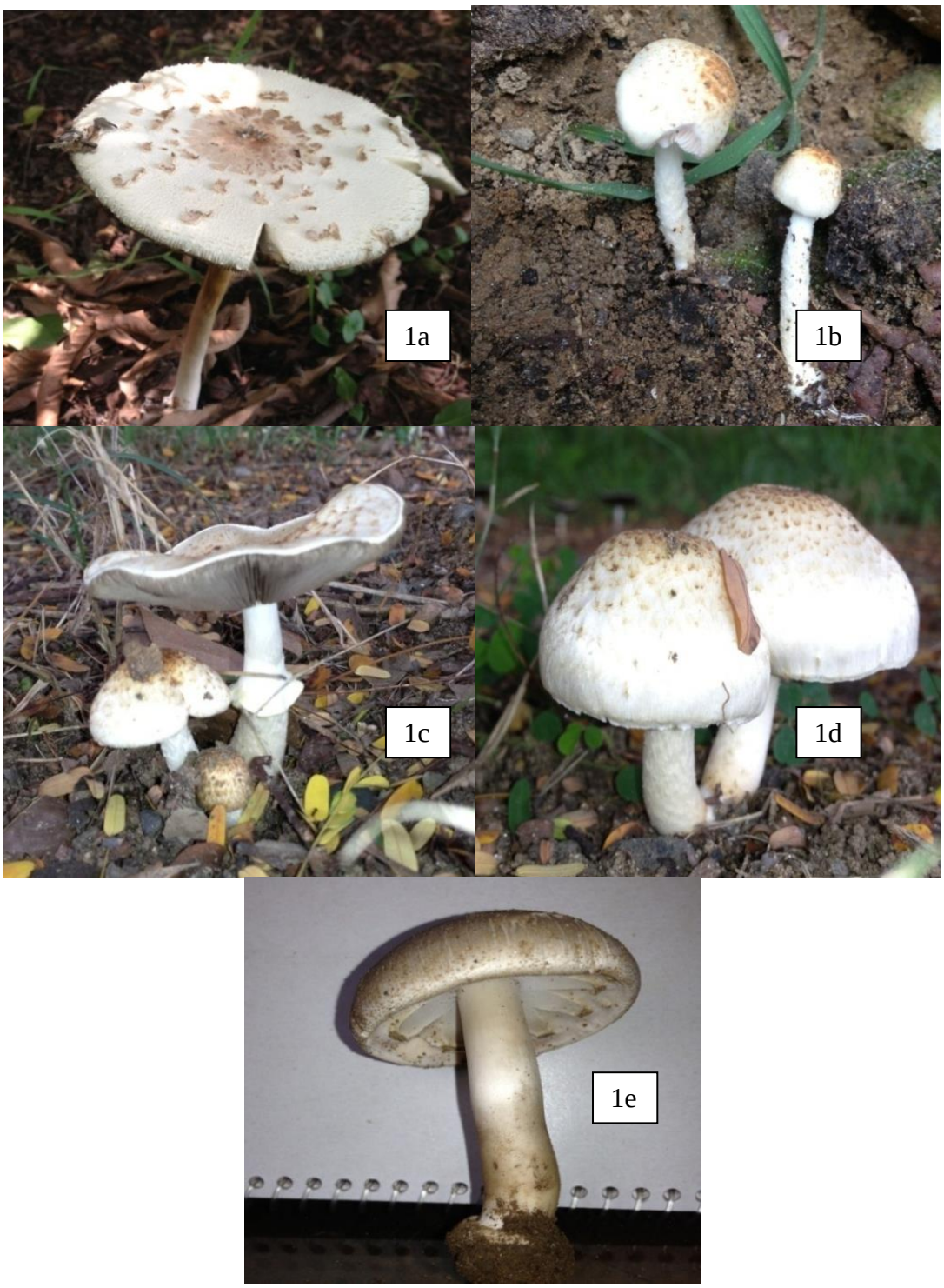

Fig. 1 − (1a − 1e) − Wild mushrooms collected from the district Ludhiana from various localities. *Chlorophyllum molybdites, Agaricus* sp., *Agaricus silvicola, Agaricus silvicola* and *Agaricus arvensis*

soil pH and moisture were 7.1 and 56%, respectively. The C, N, P, K content was also measured along with $CaCO_3$ and Fe_2O_3, from which carbon content was 5.5 mg/g of soil and nitrogen content was 1.6 mg/g of soil (Table 2). The pileus was creamish brown with 58mm diameter, which was depressed from top, scaleless and its margin was wavy. The pileus surface was non-sticky and non-hygrophanous in nature. The centrally attached stipe was also creamish colored with 50mm in length, slender shape and swollen base. An annulus type ring was present on the stipe near to pileus, but veil and volva were not present (Table 1). The lamellae underneath the pileus were freely attached to stipe with greyish coloration which later on maturation showed grayish black spores.

Table 1 Detailed account of wild mushroom samples collected from the district Ludhiana

Mushroom	DMRO-568 (1)	DMRO-569 (2)	DMRO-570 (3)	DMRO-571 (4)	DMRO-572 (5)
Collection date	10-07-2013	5-08-2013	11-09-2013	21-09-2013	22-09-2013
Location, GPS data	Ayali kalan (Ludhiana) 30° 53' 40" N 75° 45' 37" E	Hambran (Ludhiana) 30° 56' 20" N 75° 40' 13" E	Ladowal (Ludhiana) 30° 59' 28" N 75° 44' 10" E	Ladowal (Ludhiana) 30° 59' 28" N 75° 44' 10" E	Ludhiana City 30° 54' 19" N 75° 48' 51" E
Habitat, Vegetation community	Leaf litter, Road side	Cattle Dung, Road side	Leaf litter, Grass	Leaf litter, Grass	Leaf litter, Tree
Smell, Taste	Typical Mushroom, Tasteless	Typical Mushroom, Tasteless	Mushroom like, Tasteless	Mushroom like, Tasteless	Mushroom like, Tasteless
Spore print	Sea green	Brown	Brown	Brown	Brown
Pileus diameter, Color	110 mm, Creamish	23 mm, Creamish brown	58 mm, Creamish brown	50 mm, Creamish brown	49 mm, Greyish Cream
Pileus shape, Margin	Flattened, Round	Ovate, Curved	Depressed, Wavy	Ovate, Curved	Convex, Curved
Pileus Surface	Non-sticky, Non-hygrophanous	Non-sticky, Non-hygrophanous	Non-sticky, Non-hygrophanous	Non-sticky, Non-hygrophanous	Non-sticky, Non-hygrophanous
Scales	Present	Fine scales	Fine scales	Fine scales	No
Stipe attachment	Central	Central	Central	Central	Central
Stipe size, Color	205 mm, Creamish	38 mm, Creamish	50 mm, Creamish	44 mm, Creamish	45 mm, Greyish Cream
Stipe shape, Base	Slender, Swollen	Slender, Swollen	Slender, Swollen	Slender, Swollen	Slender, Swollen
Ring	Present	Present	Present	Present	Present
Veil	No	No	No	No	No
Volva	No	No	No	No	No
Basal association	No	No	No	No	No
Gill attachment	Free	Free	Free	Free	Free
Gill color, edges	Light green	Pinkish	Greyish	Creamish	Creamish, Closed
Edibility	Poisonous	Not sure	Edible	Edible	Edible
Similarity with	*Chlorophyllum molybdites*	*Agaricus* sp.	*Agaricus silvicola*	*Agaricus silvicola*	*Agaricus arvensis*

Agaricus silvicola Fig. 1d

It was collected from different location in the same locality as previous *Agaricus silvicola* during the month of September, 2013. Soil around the mushroom was collected and analyzed for physicochemical properties. The soil was assessed as sandy loam containing clay, silt and sand as 8.30, 16.3 and 55.2%, respectively. The soil pH and moisture were 7.2 and 51%, respectively. The C, N, P, K content was also measured along with $CaCO_3$ and Fe_2O_3, from which carbon content was 5.3 mg/g of soil and nitrogen content was 1.1 mg/g of soil (Table 2). This wild mushroom sample showed similar morphological characteristics to previous collected sample (3) except pileus diameter (50mm), shape (ovate) and margin (curved). The stipe size was 44mm. This wild mushroom sample could be considered as young stage of earlier discussed *Agaricus silvicola*. The lamellae underneath the pileus were freely attached to stipe with creamish coloration which on maturation showed grayish black spores (Table 1).

Agaricus arvensis Fig. 1e

Wild mushroom was collected from PAU campus, Ludhiana city on GPS location 30°54' 19" N 75° 48' 51" E grown on leaf litter underneath a tree, during the month of September, 2013. Soil around the mushroom was collected and analyzed for physicochemical properties. The soil was assessed as clayey loam containing clay, silt and sand as 8.90, 16.9 and 50.7%, respectively. The soil pH and moisture were 7.2 and 58%, respectively. The C, N, P, K content was also measured along with $CaCO_3$ and Fe_2O_3, from which carbon content was 4.8 mg/g of soil and nitrogen content was 1.7 mg/g of soil (Table 2). Smell of this wild mushroom sample was typical and taste was not distinct. The convex pileus was of greyish cream color with 49mm diameter with curved margin. Nature of pileus surface was scaleless, non-sticky and non-hygrophanous. Cream colored slender shaped stipe of 45mm length was centrally attached to pileus with swollen base. An annulus type of ring was also present but veil and volva were absent (Table 1). Gill attachment was not visible due to fusion of ring with lamellae, its color was creamish and edges were closed, but on maturation gave chocolate brown color spores.

Characterization of wild mushroom cultures

Five mushrooms collected during the survey were tissue cultured and subjected to growth studies as well as their enzyme producing capability.

Linear growth study

Linear growth of mushroom cultures was studied on complete yeast extract medium up to 10 days at 25±2 °C. The colony growth (mm/day) was observed at 4, 6, 8 and 10 day interval (Table 3). The growth was maximum for *Agaricus arvensis* (DMRO-572) as 8.00mm/d while growth was minimum for *Agaricus* sp. (DMRO-569) as 5.25mm/d, on 4^{th} day of incubation. On 6^{th} day the growth was 8.67 and 8.00mm/d for *Agaricus silvilcola-b* (DMRO-571) and *Agaricus arvensis* (DMRO-572), while on 8^{th} day the growth was maximum for *Agaricus silvicola-a* (DMRO-571) i.e., 9.12mm/d (Fig. 2).

Biomass production

The mushroom cultures were grown in CYM broth at 25±2°C for 10 days. The biomass was collected on 5^{th}, 10^{th} and dried at 55°C for 4 hours to record the biomass dry weight. On 5^{th} and 10^{th} day, the biomass was maximum for *Chlorophyllum molybdites* (DMRO-568) was 1.20g/L/d and 2.48g/L/d, respectively. The biomass was minimum for the culture of *Agaricus silvicola-b* (DMRO-571) (Table 4).

Enzyme activity of wild mushroom cultures

Mushroom cultures were grown on Mushroom minimal media broth for 10 days. The culture filtrate was collected and stored at 4 °C. Cellulases, xylanases and laccases were assayed as specific enzyme activity in U/mg total proteins (Table 5). In Cellulases, two enzymes exoglucanase and

Table 2 Physico-chemical properties of soil

Mushroom Sample no.	Physico-chemical properties Concentration in mg/g of soil											
	Soil type	**Clay (%)**	**Silt (%)**	**Sand (%)**	**Moisture (%)**	**pH**	**Carbon**	**Nitrogen**	**$CaCo_3$**	**Fe_2O_3**	**P**	**K**
Chlorophyllum molybdites	Sandy loam	8.00	16.6	53.8	54	7.5	5.2	1.3	3.0	1.5	4.5	102
***Agaricus* sp.**	Clayey Loam	9.20	15.7	51.3	57	7.3	5.9	1.5	2.7	1.7	3.8	92
***Agaricus silvicola*-a**	Sandy loam	8.70	15.2	54.5	56	7.1	5.5	1.6	2.6	2.2	4.1	85
***Agaricus silvicola*-b**	Sandy loam	8.30	16.3	55.2	51	7.2	5.3	1.1	3.2	2.0	3.2	93
Agaricus arvensis	Clayey sandy	8.90	16.9	50.7	58	7.2	4.8	1.7	2.8	1.9	4.7	97
CD (5%)	NS	NS	0.269	0.515	1.818	NS	0.181	0.181	NS	NS	0.50	5.14

Average of triplicates
Sample collected in sterile polybags
Season- Rainy (July, August, September 2013),
Temperature at that time-25−32 °C,
pH measured with standard pH-meter

Table 3 Linear growth of wild mushroom cultures

Mushroom	Linear growth (mm/day) Incubation period (d)				CD (5%)
	4d	**6d**	**8d**	**10d**	
Chlorophyllum molybdites	6.38	6.17	8.69	9.00	0.49
Agaricus sp.	5.25	5.00	5.62	6.20	0.51
Agaricus silvicola-a	5.75	5.33	6.00	6.70	0.22
Agaricus silvicola-b	7.50	8.67	9.12	9.00	0.67
Agaricus arvensis	8.00	8.00	8.75	9.00	NS
CD (5%)	0.91	0.91	0.24	1.02	

Average of three replicates
Petri plate diameter – 90 mm
Medium- Complete yeast extract medium agar, pH-6.5
Incubation Temperature – 25±2 °C
Incubation Time – 10 days

Table 4 Biomass production of wild mushroom cultures

Mushroom	Dry weight (in g/L/day) Incubation period (d)		CD (5%)
	5d	10d	
Chlorophyllum molybdites	1.20	2.48	0.32
Agaricus sp.	1.40	1.75	0.32
Agaricus silvicola-a	1.20	1.62	0.32
Agaricus silvicola-b	0.88	1.40	0.33
Agaricus arvensis	1.08	1.61	0.45
CD (5%)	0.28	0.16	

Average of three replicates
Weight of Dry filter paper– 1.1 g
Medium- Complete Yeast Extract Medium (CYM), pH-6.5
Incubation Temperature – 25±2 °C
Incubation Time – 10 days
Drying temp. and time - 55°C , 4 hours

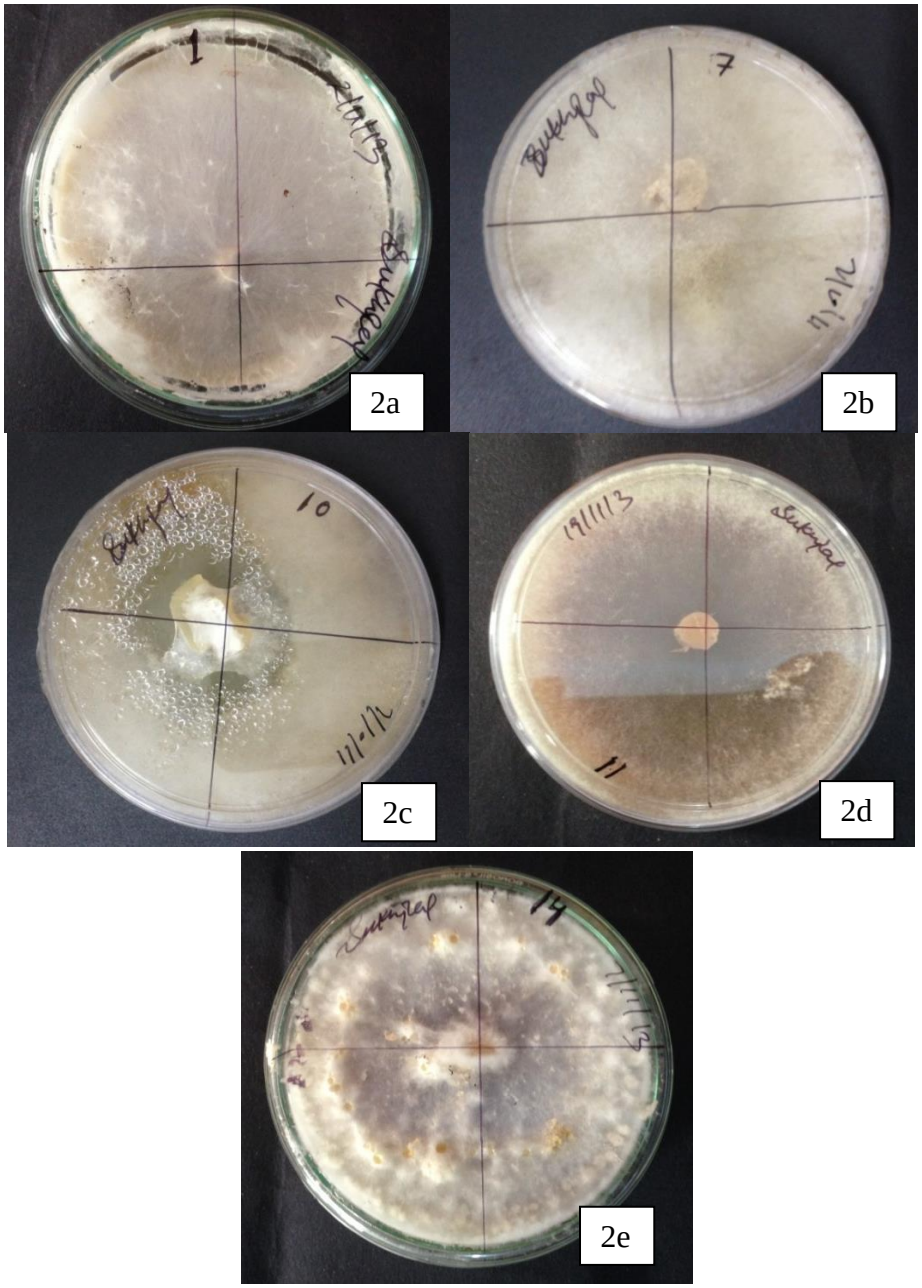

Fig. 2 − Linear growth (2a − 2e) − *Chlorophyllum molybdites, Agaricus* sp., *Agaricus silvicola-a, Agaricus silvicola-b* and *Agaricus arvensis.*

endoglucanase were estimated. The exoglucanase activity was maximum for *Agaricus silvicola*-a (DMRO-570) as 1.468 U/mg proteins, followed by *Agaricus silvicola*-b (DMRO-571) as 0.852 U/mg proteins whereas endoglucanase activity was maximum for *Agaricus silvicola*-b (DMRO-571), *Agaricus silvicola*-a (DMRO-570) and followed by *Agaricus* sp. (DMRO-569). The xylanase activity was maximum for *Agaricus silvicola*-b (DMRO-571) as 1.622 U/mg proteins. The laccase activity was 5.25 U/mg proteins for *Agaricus* sp. and *Agaricus arvensis*.

Table 5 Enzyme activity of wild mushroom cultures

	Specific enzyme activity (U/mg protein)			
Mushroom	**Exoglucanase**	**Endoglucanase**	**Xylanase**	**Laccase**
Chlorophyllum molybdites	0.143	0.175	0.506	3.25
Agaricus sp.	0.522	1.289	0.138	5.25
Agaricus silvicola-a	1.468	1.417	0.779	1.03
Agaricus silvicola-b	0.852	1.622	0.448	2.01
Agaricus arvensis	0.516	0.568	0.383	5.25
CD (5%)	0.200	0.327	0.245	0.361

Average of three replicates Incubation temperature-25±2°C, Incubation time-10 days
Medium used- Mushroom minimal medium, pH-6.5
Wavelength-Exoglucanase, endoglucanase and xylanase was 540nm(Sandhu & Kalra 1982).
For Laccase 495nm (Dhaliwal et al. 1991)
Reference-For Endoglucanase, Exoglucanase and Laccase standard glucose solution used.
For xylanase standard xylose solution used.

Growth rate during spawn preparation

Mushroom cultures were grown on wheat grain substrate to study their growth potential for spawn production. The observations were made at 25±2 °C up to 24 days. The growth (mm/day) was recorded on 8th, 16th and 24th day of incubation (Table 6). On 8th day, maximum growth was observed for cultures *Agaricus arvensis* (DMRO-572) as 4.25mm/d, while on 16th day maximum growth rate was observed in *Chlorophyllum molybdites* (DMRO-568). On 24th day five cultures showed growth at par in cultures *Chlorophyllum molybdites* (DMRO-568) and *Agaricus arvensis* (DMRO-572) (Fig. 3).

Table 6 Spawn production from wild mushroom cultures on wheat grains

Mushroom	**Mycelial run rate (in mm/day) Incubation period (d)**			**CD (5%)**
	8d	**16d**	**24d**	
Chlorophyllum molybdites	1.88	4.19	5.69	0.29
Agaricus sp.	1.50	3.31	4.54	0.58
Agaricus silvicola-a	1.75	3.38	4.79	0.29
Agaricus silvicola-b	1.25	3.06	4.25	0.53
Agaricus arvensis	4.25	3.69	5.66	0.44
CD (5%)	0.59	0.20	0.31	

Average of three replicates Incubation Temperature – 25±2 °C
Test tube size- (25mm×198mm) Incubation Time – 24 days
Substrate- Boiled wheat grains

Substrate selection

In order to study the feasibility of growth of wild mushroom cultures on wheat straw, paddy straw and wheat straw based compost were used as substrates. The growth of cultures was observed up to 24 days (Table 7). Out of five cultures, *Chlorophyllum molybdites* (DMRO-568) and *Agaricus arvensis* (DMRO-572) showed consistently better growth on compost as observed on 8th, 16th as well as on 24th day. On wheat straw, the growth rate was higher on 8th and 16th day for the culture *Agaricus arvensis* (DMRO-572), while on 24th day it showed maximum growth with DMRO-568 as well. On paddy straw, maximum growth rate was observed from the culture *Agaricus arvensis* (DMRO-572) (Fig. 4).

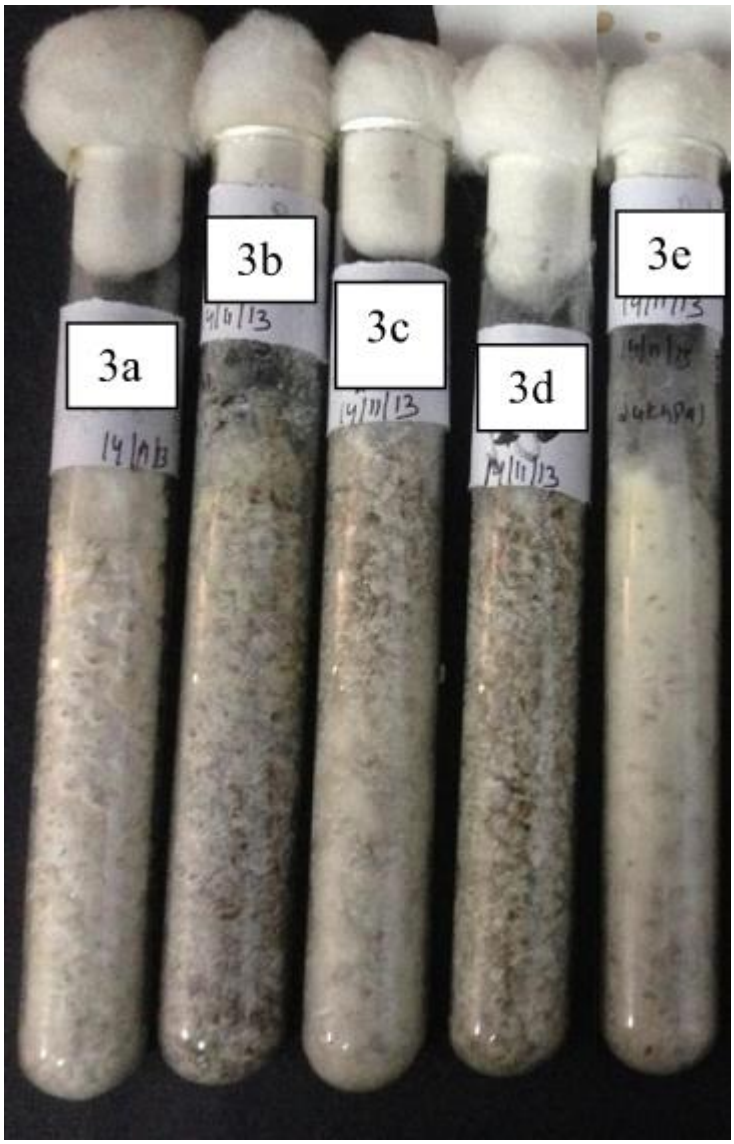

Fig. 3 − Spawn preparation of wild mushroom cultures (3a − 3e) − *Chlorophyllum molybdites, Agaricus* sp., *Agaricus silvicola-a, Agaricus silvicola-b* and *Agaricus arvensis.*

Table 7 Substrate selection to study possible fruiting of wild mushroom cultures

Mushroom	**Growth rate(in mm/day) Incubation period (d) Substrates**								
	Compost			**Wheat straw**			**Paddy straw**		
	8d	**16d**	**24d**	**8d**	**16d**	**24d**	**8d**	**16d**	**24d**
Chlorophyllum molybdites	2.25	1.59	3.75	2.75	1.78	2.06	2.25	1.31	1.10
Agaricus sp.	3.69	2.00	1.54	2.00	1.34	1.10	0.00	0.00	0.00
Agaricus silvicola-a	0.00	0.00	0.00	1.44	0.78	0.62	0.00	0.00	0.00
Agaricus silvicola-b	2.12	1.19	1.06	1.94	1.56	1.67	2.12	1.19	1.12
Agaricus arvensis	2.12	1.47	3.00	2.44	1.28	3.75	2.94	5.62	3.75
CD (5%)	Factor A-0.12, Factor B-0.07, Factor C-0.07, A×B-0.21, A×C-0.21, B×C-0.12, A×B×C-0.37								

Average of three replicates
Petri plate diameter – 90 mm
Substrates-Compost, wheat straw, paddy straw
Incubation Temperature – 25±2°C
Incubation Time – 24 days

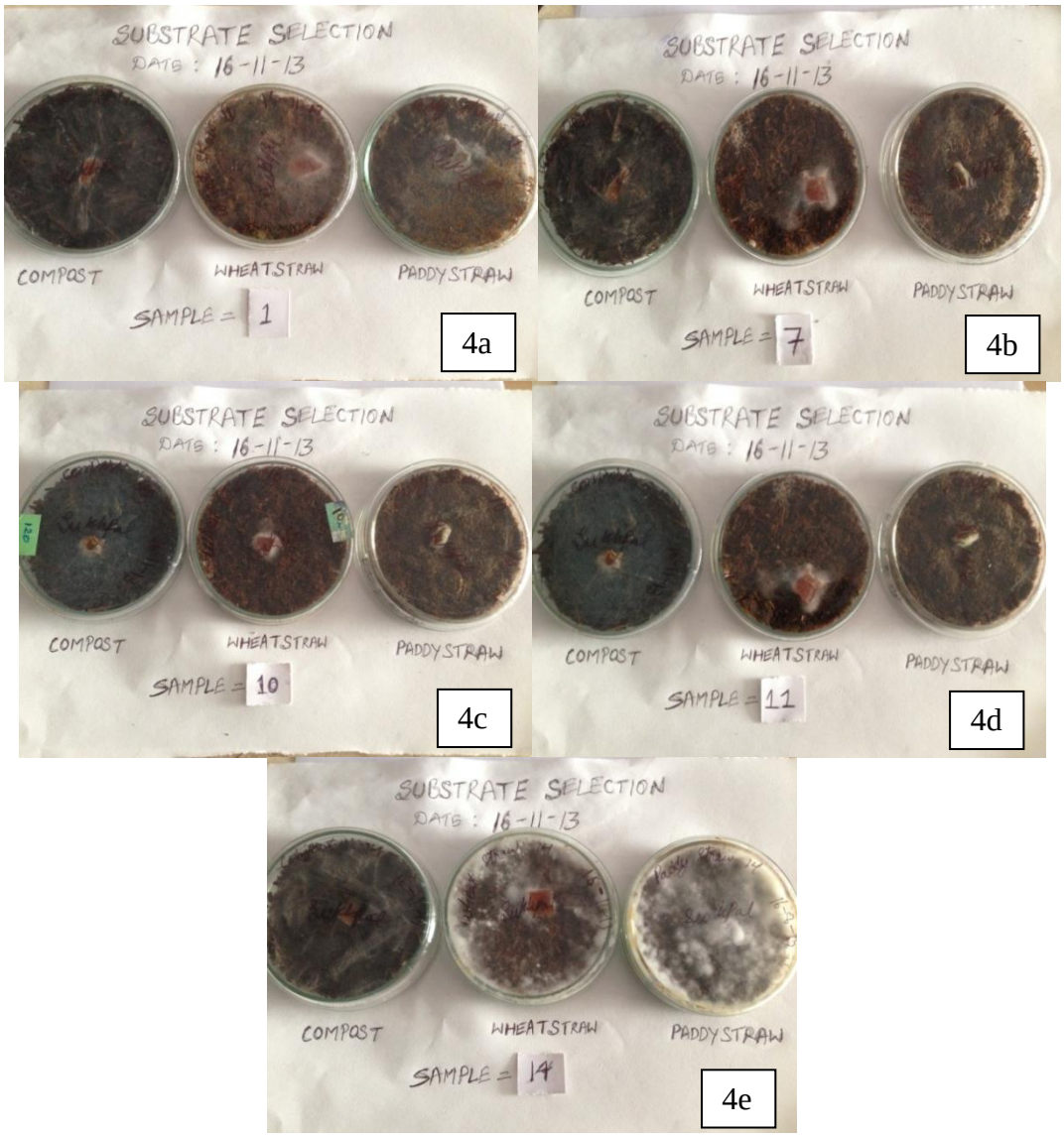

Fig. 4 − Substrate selection for wild mushroom cultures (4a − 4e) − *Chlorophyllum molybdites*, *Agaricus* sp., *Agaricus silvicola-a*, *Agaricus silvicola-b* and *Agaricus arvensis.*

Discussion

Chlorophyllum molybdites

C. molybdites earlier showed morphological similarity with *Lepiota brunnea* or *Lepiota rachodes* which are edible species. However, the green spore print distinguished it to put under the genus *Chlorophyllum* and species *molybdites* (Kumar & Kaviyarasan, 2012). On the basis of this morphological characterization mushroom was identified as *Chlorophyllum molybdites* with taxonomic position: Kingdom: Fungi, Division: Basidiomycota, Class: Agaricomycetes, Subclass: Homobasidiomycetidae, Order: Agaricales, Family: Agaricaceae, Genus: *Chlorophyllum*, Species: *molybdites* as confirmed by the DMR Scientists.

Agaricus sp.

Pinkish lamellae during younger stage of fruit body which on maturation turned brown and pileus color, shape showed its near relationship with *Agaricus bisporus* also reported by Karwa & Rai, 2010. So, identified mushroom was related to the genus *Agaricus* with taxonomic position, Kingdom: Fungi, Division: Basidiomycota, Class: Agaricomycetes, Order: Agaricales, Family: Agaricaceae, Genus: *Agaricus.*

***Agaricus silvicola*-a**

The characters (Table 1) were nearly similar to species of genus *Agaricus* and species *silvicola* as reported and discussed by Al-Momany & Saleh, 2009. On these bases taxonomic position of this mushroom is Kingdom: Fungi, Division: Basidiomycota, Class: Agaricomycetes, Order: Agaricales, Family: Agaricaceae, Genus: *Agaricus,* species: *silvicola.*

***Agaricus silvicola*-b**

Morphologically (Table 1) it showed characteristics close to *Agaricus silvicola* as reported earlier (Al-Momany & Saleh 2009). It can also be considered as the young stage of *Agaricus silvicola*-a, but their samples were collected separately from different places in same village. So, the taxonomic position of this macrofungus is, Kingdom: Fungi, Division: Basidiomycota, Class: Agaricomycetes, Order: Agaricales, Family: Agaricaceae, Genus: *Agaricus*, species: *silvicola.*

Agaricus arvensis

The features were pointing towards similarity of this sample with the genus *Agaricus* and species *arvensis* (Karwa & Rai 2010). On these bases the taxonomic position of *Agaricus arvensis* is Kingdom: Fungi, Division: Basidiomycota, Class: Agaricomycetes, Order: Agaricales, Family: Agaricaceae, Genus: *Agaricus,* species: *arvensis.*

Linear growth study

Radial growth comparison have been reported earlier between wild mushrooms, *Russula* sp. and *Pycnoporus cinnabarinus*. From both *Russula* sp. showed faster growth rate and petri-plate was full before the completion of 3rd day of incubation but *Pycnoporus cinnabarinus* showed 9mm/d growth up to 7th day of incubation (Shittu et al. 2005). The wild varieties showed linear growth rate in appropriate range as our results observed from *Agaricus arvensis, Agaricus silvicola*-b and *Chlorophyllum molybdites* on 4th, 6th, 8th and 10th day of incubation (Table 3). Statistical analysis of linear growth study showed significant growth rate increment in all cultures along with different incubation time intervals except *Agaricus arvensis*, which showed steady growth rate. Growth rate along different cultures showed significant deviation between their mycelium extension rates.

Biomass producing capability

The biomass production showed similar pattern of maximum growth as that of linear growth (Table 3). An experiment performed to know effect of different carbon and nitrogen sources on the biomass production of *Auricularia polytricha* recorded maximum growth by using glucose and sucrose as carbon source to show 4.52 and 3.15g/L biomass after 15 days of incubation. With respect to nitrogen source, it showed maximum biomass (3.94g/L) by using yeast extract (Hassan and Ghada 2012). *Chlorophyllum molybdites* showed result for biomass production in Complete yeast extract broth in comparable to the growth results obtained from *Auricularia polytricha,* after 10 days of incubation (Table 4). These cultures expressed significant progress in biomass production along two different incubation periods within a sample. They also showed significant difference in their biomass production rate comparatively.

Enzyme activity

Agaricus silvicola (a-b) showed maximum exoglucanase and endoglucanase activity due to the reason of its relation with the genus *Agaricus,* which is a known lignocellulolytic fungus. Wild isolates of *Agaricus bisporus* were collected from various habitats for studies on their adaptation to the colonization of conventional mushroom. Twenty field isolates from two distant sites and 6 cultivars had been studied to produce 11 extracellular enzymes. The isolates with highest mycelial growth rates were those that produced balanced pools of enzyme activities (Savoie et al. 1996). So, due to this the fast growing cultures observed during linear and biomass production studies (Table 3 and 4) showed more enzyme activity (Table 5). Analysis of specific enzyme activities of these

cultures by CPCS1 showed significant variation in lignocellulolytic enzyme producing capabilities, though most of these were related to the same genus.

Spawn preparation

Stanley and Awi-waadu (2010) had studied different grains to check mycelial extension rate. Growth of mycelia of *P. tuber-ragium* was observed on white maize grains as 3.76 mm/day and on wheat grains as 1.57 mm/day. Growth rate of *Pleurotus giganteus* on solid media like brown rice grain, corn grain, Job's tear grain, black kidney bean, mung bean, rye grain, sorghum grain, soy bean and wheat grain was observed with rate on wheat grains as 7.74mm/d (Kumla et al. 2013), which was comparable to the observations taken from wild mushrooms *Chlorophyllum molybdites* (DMRO-568) and *Agaricus arvensis* (DMRO-572) (Table 6). Acceptability of these mushroom cultures towards wheat grains was also different and statistical analyses of this data significantly support this view.

Substrate selection

The growth characteristics (lag time after inoculation, colonization period, extension rate) of five *L. edodes* strains has been evaluated in 'race-tubes' filled with oak wood saw dust, wheat straw, corn cobs and cotton waste (Philippoussis et al. 2002). The growth rates in the range of 4.92 – 3.38 mm/day was recorded and took only 30, 37, 46 and 52 days to colonize OS, WS, CC and CW, respectively (Philippoussis et al. 2002). The wild mushroom cultures *Agaricus arvensis* and *Chlorophyllum molybdites* showed similar range of growth rate on compost, wheat and paddy straw (Table 7). Statistical analysis showed significant effect of different substrates and incubation intervals on the growth of wild mushroom cultures.

Conclusions

It can be concluded that four species related to well known genus *Agarics* have been collected with a commercial edible potential. But *Chlorophyllum molybdites* is inedible. These basidiomycetes have been reported first time from the district Ludhiana. Tissue culturing and growth characteristics study of these wild mushrooms could help to collect, preserve and analyze a known macrofungal livestock for future commercialization. This study also indicated that good macrofungal diversity is present in the middle region of Punjab plains, whose surveillance could provide more options of wild mushroom varieties to fulfill the needs of mankind.

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